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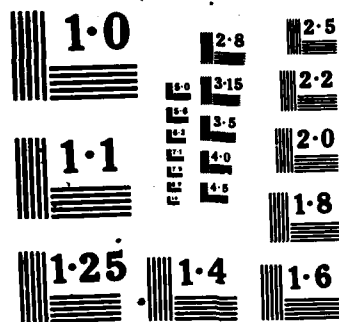
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SPECTROSCOPIC STUDIES OF MELANIN

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Spectroscopic studies of melanin--Cooper et al

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excited by argon ion and helium-neon laser radiation. The full width at half maximum, position, and intensity of fluorescence were measured. The full width at half-maximum (3600 cm^{-1}) was independent of excitation wavelength. At higher energy excitation, the emission maximum was independent of excitation line. At lower energy excitation, the emission maximum varied with the excitation line. The emission intensity dropped by a factor of 6 as the excitation wavelength was varied from 465.8 nm to 514.5 nm. Luminescence was also observed upon excitation at 632.8 nm of melanin dissolved in DMSO. Raman data, obtained from solid melanin suspended in a KBr pellet (rotating sample cell, 488.0 nm excitation, 5 cm^{-1} band pass), revealed broad bands at 1385 cm^{-1} , $1590\text{--}1615\text{ cm}^{-1}$ and 2930 cm^{-1} superimposed upon a weak fluorescent background. Upon ultraviolet laser excitation (363.8 nm, 5 mW power on the sample, 5 cm^{-1} band pass), a single broad band appeared, centered at 1300 cm^{-1} . The difference from visible excitation implies that resonance enhancement from an ultraviolet absorbing chromophore occurred.

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ABSTRACT

→ To gain insight into the photochemistry of melanin, we have measured chemiluminescence and Raman spectra of synthetic tyrosine melanin. Chemiluminescence kinetics were obtained from a sealed capillary tube containing a basic melanin solution. Excitation at 488.0 nm and monitoring emission at 633 nm produced a first order rise and a second order decay in intensity to steady state levels. We collected steady state emission spectra from a flow system excited by argon ion and helium-neon laser radiation. The full width at half maximum, position, and intensity of fluorescence were measured. The full width at half-maximum ($3600/\text{cm}^{-1}$) was independent of excitation wavelength. At higher energy excitation, the emission maximum was independent of excitation line. At lower energy excitation, the emission maximum varied with the excitation line. The emission intensity dropped by a factor of 6 as the excitation wavelength was varied from 465.8 nm to 514.5 nm. Luminescence was also observed upon excitation at 632.8 nm of melanin dissolved in DMSO. Raman data, obtained from solid melanin suspended in a KBr pellet (rotating sample cell, 488.0 nm excitation, $5/\text{cm}^{-1}$ band pass), revealed broad bands at $1385/\text{cm}^{-1}$, $1590-1615/\text{cm}^{-1}$ and $2930/\text{cm}^{-1}$ superimposed upon a weak fluorescent background. Upon ultraviolet laser excitation (363.8 nm, 5 mW power on the sample, $5/\text{cm}^{-1}$ band pass), a single broad band appeared, centered at $1300/\text{cm}^{-1}$. The difference from visible excitation implies that resonance enhancement from an ultraviolet absorbing chromophore occurred. ↑

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PREFACE

The authors thank Mr. Earl McMasters and Dr. Aaron Lewis of the Department of Engineering and Applied Science, Cornell University, Ithaca, N.Y. 14853, for use of their Raman facility; Dr. Brian M. Pierce for assistance in designing proper controls for the Raman experiments; Mr. David J. Lund for alignment and operation of the laser optics; Mr. Thomas Haw; Dr. James Gallas; Ms. Christine L. Noah-Cooper for stimulating and useful conversations; and Lottie B. Applewhite for editorial assistance.

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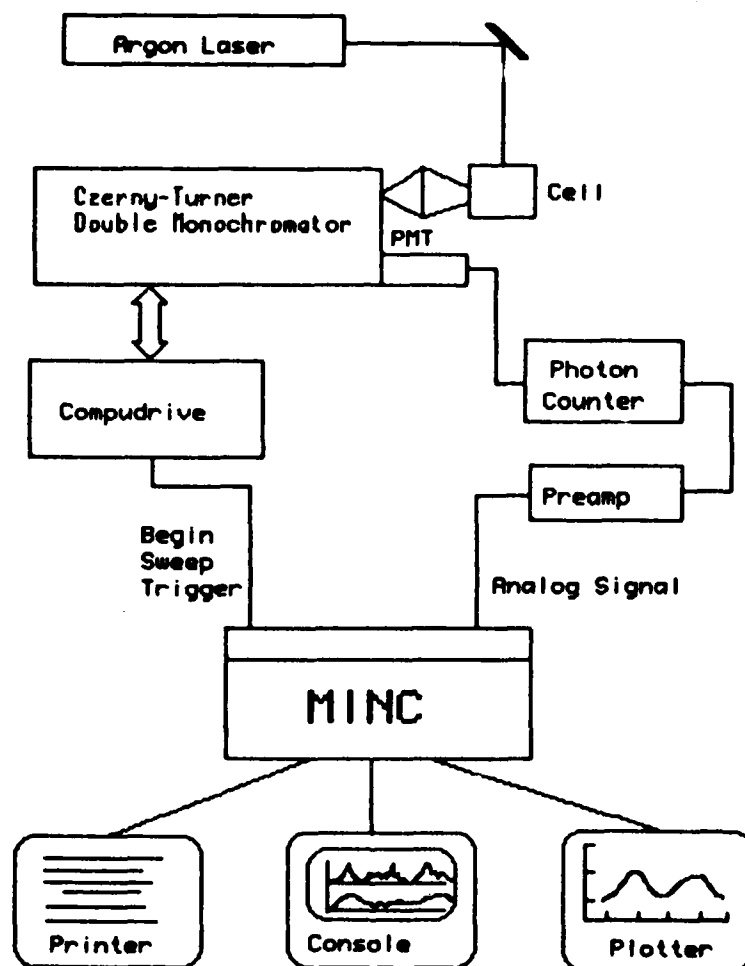
SPECTROSCOPIC STUDIES OF MELANIN

Melanins are polymers found throughout nature. They are found in skin pigment, hair, eyes, the inner ear, and in the brain. A knowledge of the spectroscopic properties of melanin is essential for an understanding of its biological function. Melanin is found in areas where a high energy flux occurs. In the skin it protects against damage by ultraviolet (UV) light (1). In the inner ear it protects the sound receptor cells against sound damage (2). In the eye it absorbs UV and stray light thereby preventing eye damage and photophobia (3). Melanin is found in the substantia nigra of the brain (4) where it is thought to serve as an energy transfer acceptor to excited species produced by metabolism (5), (6). It is thought that damage to neuromelanin may lead to Parkinsonism (7). Because melanin behaves as a general purpose energy absorber in biological systems, an understanding of its responses to energy absorption will shed light on its role in living systems. Spectroscopic investigations will gain information about the dynamics of energy transduction in melanin, and will lend insight into its photochemical properties.

The absorption spectrum of melanin fits to an amorphous semiconductor model (8). The majority of spectroscopic studies of melanin have taken advantage of the presence of free radicals under all conditions. Sealy et al. (9) have extensively studied melanin using electron spin resonance spectroscopy. The electron spin resonance results have shown melanin to be photosensitive, increasing its free radical concentration under illumination (10), (11), and leading to the production of oxygen radicals (12). Gallas (13) and Kozikowski et al. (14) have studied melanin fluorescence. As part of a long-term spectroscopic investigation of melanin, in this paper we report luminescence and Raman data obtained from irradiation of melanin samples by argon ion laser radiation. Since melanin fluoresces under most conditions, we have studied its fluorescent properties in detail. We deduced a general mechanism of photochemistry from chemiluminescence data; more importantly we optimized the experimental conditions for the first observation of Raman scattering from melanin and assigned the prominent bands in the vibrational spectrum.

MATERIALS AND METHODS

Melanin, synthesized from the persulfate oxidation of tyrosine, was obtained from Sigma chemical company and used without further purification. The samples were illuminated by an argon ion laser (Spectra Physics-164-01). Figure 1 shows a schematic diagram of the



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Figure 1. Diagram of apparatus used in this study. An argon ion laser excites the sample, a flow cell or a rotating solid sample. The emitted or scattered light enters a Czerny-Turner double monochromator where the light intensity is measured by photoncounting electronics. The monochromator is controlled by a Spex Industries CD2A compudrive. The compudrive also initiates signal acquisition by sending a trigger pulse to a computer (Nicolet MED 80 or Digital Equipment Corporation MINC).

apparatus. To demonstrate the existence of photoreactions in melanin, a sample of melanin was placed in a capillary tube and illuminated at 488.0 nm at a power of 90 mW at the laser head. The intensity of light produced was monitored at 633 nm and chemiluminescence kinetics were collected with a Kipp and Zonen chart recorder. The time constant of the apparatus was 100 msec. When a steady state intensity of luminescence was obtained, the tube was quickly moved forward 2 mm such that a new region of the sample was placed in the beam. Kinetic data were collected until the chemiluminescence intensity again reached a steady state level.

Because of the complex light-induced chemical reactions in melanin observed by Slawinski (15), (16), a flow system was devised such that a melanin solution in a reservoir (100 ml, 0.5 mg/ml, 0.1 N NaOH) was pumped through a 1.85 mm diameter capillary tube by a peristaltic pump and then recirculated into the reservoir. This flow method minimized photodegradation of the sample. Before spectra were collected, the chemiluminescence intensity was monitored to ensure that a steady state luminescence had been reached. The intensity of fluorescence was normalized to the laser power at the lines employed.

Raman spectra were collected from melanin that had been pressed into KBr pellets. Melanin was mixed with KBr at a concentration of 0.6% (w/w) with a mortar and pestle. The mixture was then subjected to 20,000 lb pressure, forming a 13 mm diameter pellet. The pellet was placed on a sample rotator and the pellet rotated at 1000 rpm in order to minimize photodegradation of the sample. The sample and rotator were placed in a Spex industries model 1459 illuminator. The scattered radiation was aligned and focused into a Spex industries model 1403 double monochromator. Because the signal to noise ratio of the Raman scattering was a very sensitive function of the sample's geometry, the method of optimizing the sample geometry is presented here. The image of the scattered radiation off the sample provided a method for optimizing the signal to noise ratio. When the scattered light image of the sample had a rough, grainy appearance, the focal point of the laser beam was directly on the surface of the KBr pellet. This image was then focused to a point at the entrance of the double monochromator. Slight alterations in these initial conditions led to a large decrease in the signal to noise ratio. The rotating sample was illuminated at 488.0 nm at an input power at the laser head of 1000 mW. The estimated power on the sample was 100 mW. The scattered light, before entering the monochromator, went through a polarization scrambler converting the plane-polarized scattered radiation to circularly-polarized radiation, cancelling variations in spectrometer response resulting from polarization-dependent grating efficiencies.

The detector was an RCA C31034 photomultiplier tube cooled to -30° C in a Products for Research model TE104RF-002 thermoelectric detector chamber, with cooling water supplied by a Lauda RM6 circulating water bath (thermostat set at 15° C). The intensity data were converted to

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an analog output by a Spex DPC-2 digital photometer.

The double monochromator was controlled by a Spex industries CD2A compudrive. The compudrive also controlled the Nicolet Med-80 signal averager. The compudrive was set on a repetitive sweep mode. As a switch in the compudrive closed, a Tektronix PG 505 pulse generator was activated, sending a trigger pulse to the Med-80, initiating signal averaging. The analog output signal from the photon counter was amplified by a Tektronix 502 differential amplifier before entering the Med-80.

The Raman data were collected by averaging multiple sweeps through the use of a Nicolet Med-80 signal averager. Collection of data was done either from 600-2000 cm^{-1} or from 2000-4000 cm^{-1} (Scan rate = 10 $\text{cm}^{-1}/\text{sec}$, instrument time constant = 0.1 sec, band pass = 5 cm^{-1} , spectrum is average of 30 sweeps). To measure the relative intensities of peaks in the two domains, a low resolution spectrum was also collected from 600-4000 cm^{-1} . The long time needed to collect the data in the broader range allowed for fewer sweeps (about 15) and a lower signal to noise ratio, but an intensity ratio between the two spectral domains could be measured in order to calculate a normalized Raman spectrum of melanin.

Ultraviolet Raman spectra of melanin samples were obtained at the Raman spectrometer at Cornell University. A KBr pellet containing melanin was excited at 363.8 nm with an argon ion laser and data collected from 1000 to 3562 cm^{-1} . The spectrometer was in a stepping mode, 2 cm^{-1} step size, 50 sec integration time, 5 mW laser power on the sample and a 5 cm^{-1} band pass.

RESULTS

Figure 2 shows typical chemiluminescence kinetics obtained from a sealed capillary tube of melanin. The chemiluminescence kinetics observed were superimposed upon a steady state background. Under these conditions the luminescence intensity increased 40% upon illumination of a new region of the sample. This additional increase in illumination, resulting from the production of a phototransient, was directly proportional to the laser power illuminating the sample. The steady state background increased in the same manner. To gain insight into the mechanism of this photoreaction, the data were analyzed with the use of the Noyes equation, given in Equation 1 (17),

$$\log(t_{1/2}) = \log(f) - (n-1)\log I_0 \quad 1$$

where $t_{1/2}$ is the half life of the reaction, f is a constant, n is the reaction order and I_0 is the chemiluminescence intensity at the point from which the half-life was measured. The inset of Figure 2 is a plot of $\log(t_{1/4})$, the time needed for the chemiluminescence intensity to drop by a factor of 4 versus the $\log(I_0)$, the initial intensity from

which $t_{1/4}$ was measured. If these kinetics are second order, $t_{1/4}$ will be the half-life of the reaction (light intensity is a measure of reaction rate) and the slope of the line will be -1. If the chemiluminescence decay is first order, then the plot will have a slope of 0. In the inset of Figure 2, the slope of the plot is -1.07 ($n=10$, $r=-0.9763$), indicating that the chemiluminescence data presented in Figure 2 resulted from a second order process.

Because of the complex kinetics observed in a capillary tube, emission data were obtained with steady state measurements from a flow system. The overall chemiluminescence intensity was a function of the flow rate, illumination time, laser line used, and the laser power used. Optimum conditions for spectral measurement were obtained from a moderate flow rate (0.13 ml/sec), low laser power (<100 mW), and collection of data approximately one hour after commencement of flow and illumination. The hour wait was required to stabilize the signal. With these conditions, a portion of melanin resided in the beam less than 100 msec.

Figure 3 presents examples of typical luminescence spectra obtained with this system. The full width at half maximum (3600 cm^{-1}) was independent of the wavelength of illumination from 457.5 nm excitation to 514.5 nm excitation. Also, Figure 3 presents a luminescence spectrum of melanin dissolved in DMSO and illuminated at 632.8 nm. It is clear that the full width at half maximum is independent of excitation wavelength and only the wavelength of maximum fluorescence intensity varies with excitation wavelength.

Figure 4 is a plot of the wavenumber of maximum fluorescence versus the laser excitation wavenumber. We see that at low energy of excitation the emission maximum is a function of the excitation wavelength. As the excitation energy approaches 21839 cm^{-1} , the position of the emission maximum becomes independent of the excitation line. This trend does not continue at higher energy excitation, as the emission spectrum becomes a combination of several emitting species (D. L. Bolton, unpublished results). Figure 5 is a plot of the emission intensity at the wavelength of maximum intensity for each excitation line. The data presented here is not a true excitation spectrum where the emission wavelength is held constant and the excitation wavelength is varied. This approach was chosen because the emission maximum changed with the excitation line, while a true excitation spectrum requires that the shape of the emission spectrum not change with excitation line. The rate of increase of fluorescence is substantial. In a small energy range (19435 cm^{-1} to 21468 cm^{-1}), the fluorescence intensity increases by a factor of nearly 6. This increase in intensity was especially noticeable in the spectral region where the emission maximum does not change with excitation line. The absorbance increases by a factor of 1.27 in this wavelength domain (8).

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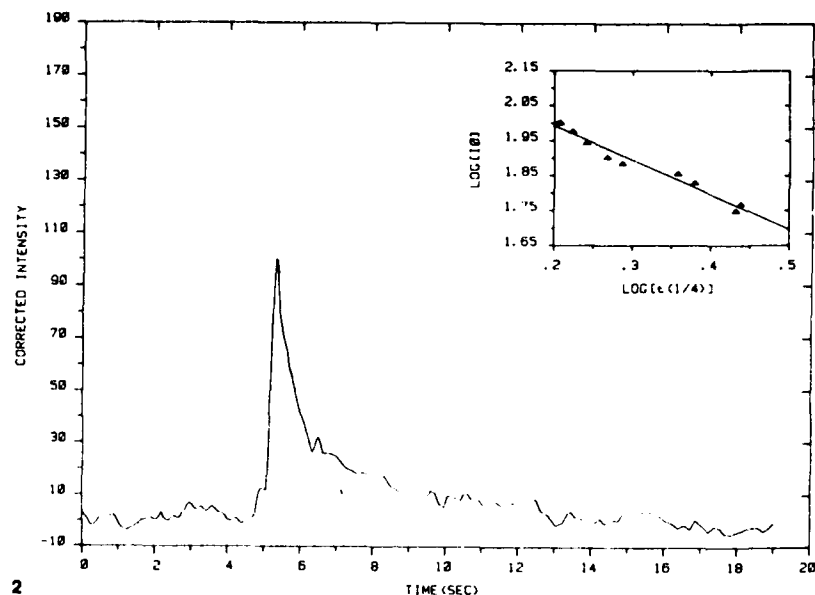


Figure 2. Chemiluminescence kinetics of melanin solution sealed in a capillary tube. A capillary tube of melanin in 0.1N NaOH was placed in an argon ion laser beam and excited at 488.0 nm. The sample was then quickly moved 2 mm and the chemiluminescence kinetics collected. The luminescence was monitored at 633 nm and the data collected on a strip chart recorded. Inset: plot of the time required for chemiluminescence intensity to decay by a factor of 4 ($t_{1/4}$) versus the light intensity (I_0) from which the $t_{1/4}$ value was measured.

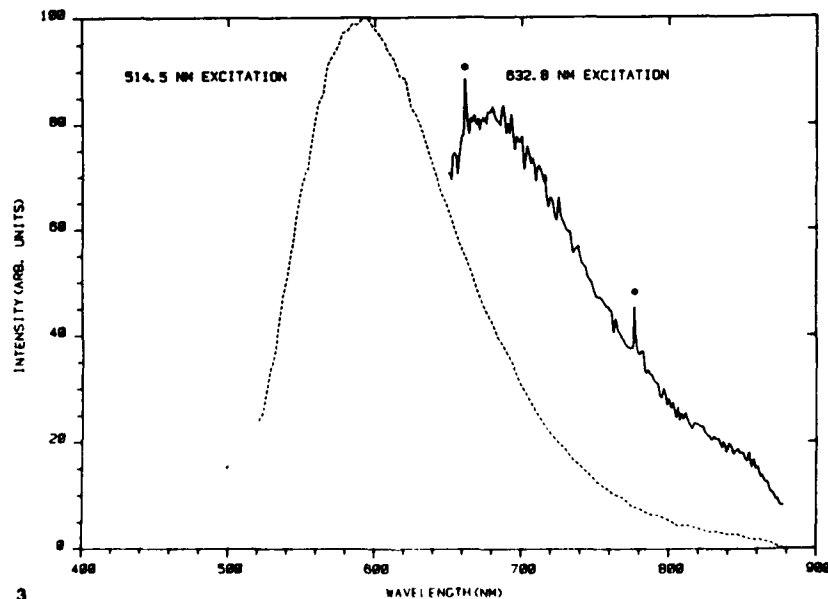


Figure 3. Chemiluminescence spectra of melanin. The solid curve is that obtained upon exciting the sample in the flow system described in the materials and methods section at 514.5 nm. The dotted curve is a chemiluminescence spectrum of melanin dissolved in DMSO when the sample was excited at 632.8 nm. The peaks marked with an asterisk are Raman bands of DMSO.

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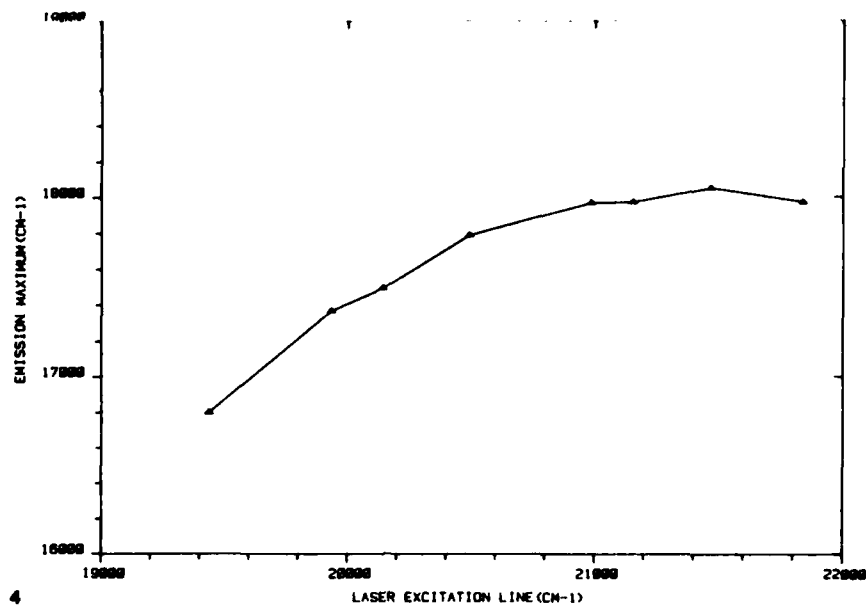


Figure 4. Plot of the wavenumber of maximum fluorescence versus the laser excitation wavenumber using the flow system.

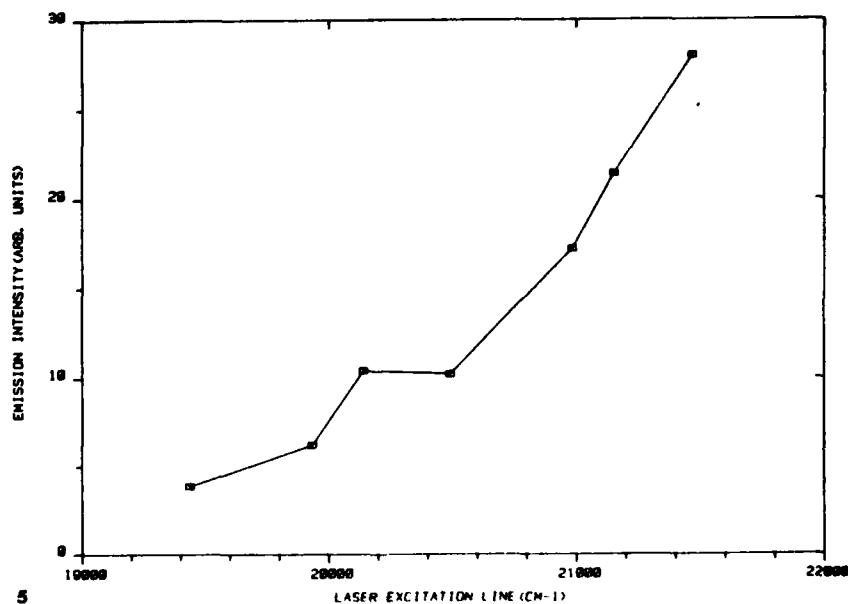


Figure 5. Plot of fluorescence intensity at the wavelength of maximum fluorescence versus the excitation wavenumber. The scatter in intensity results from experimental error.

Spectroscopic Studies of Melanin

Collection of Raman data on melanin has been hampered by the intrinsic fluorescence of melanin in aqueous solution. As has been presented in the data above, fluorescence of melanin in solution is broad and exists at all excitation wavelengths, even at 632.8 nm. The fluorescence was partially quenched through the addition of iodide ion. However, a population of the fluorophores is not accessible to the quencher (8). Even in the presence of high iodide concentration, the fluorescence intensity was high enough to make Raman scattering unobservable in aqueous solution by continuous wave techniques. As was also observed by Kozikowski et al. (14), the intrinsic fluorescence of melanin in a KBr pellet was low enough to allow the observation of Raman scattering from melanin.

Figure 6 presents typical Raman data obtained from illumination of a melanin sample pressed into a KBr pellet. The concentration of melanin in the pellet was critical for success in collecting Raman data. When a pure melanin pellet was irradiated, the sample tended to overheat to incandescence. A concentration of 0.6% (w/w) was high enough to present an adequate signal to noise ratio, but allowed for dissipation of heat from laser irradiation. No changes were observed in the appearance of the spectrum over irradiation time, implying that photodegradation was minimal under these conditions. In the region from 1000 cm^{-1} to 2000 cm^{-1} there are two broad bands, one centered at 1385 cm^{-1} , the other peaking at $1590\text{--}1615\text{ cm}^{-1}$. From 2000 cm^{-1} to 4000 cm^{-1} a broad band appears with a maximum at 2930 cm^{-1} . The Raman bands were superimposed upon a broad, weak luminescence. To show that the observed data were actually Raman scattering from melanin, we did measurements of the Raman data at more than one laser wavelength. The same spectrum was observed at 514.5 nm excitation as at 488.0 nm excitation. Also, Raman data were collected from a KBr pellet with no melanin present. No Raman scattering was observed from KBr in the spectral regions where Raman peaks were observed in the presence of melanin.

Figure 7 presents a more detailed look at the Raman spectrum of melanin. Spectrum A shows the Raman bands from $600\text{--}2300\text{ cm}^{-1}$. Not only are there two major bands at 1385 cm^{-1} and 1615 cm^{-1} , there are also unresolved bands at 1250 cm^{-1} , and 1750 cm^{-1} . Spectrum B shows the higher frequency spectral domain, where the maximum Raman scattering occurs at 2935 cm^{-1} and a shoulder appears at the vicinity of 3170 cm^{-1} .

Figure 8 is a Raman spectrum of melanin excited at 363.8 nm. It is considerably different from that obtained from excitation at 488.0 nm. A single broad band appears centered at 1301 cm^{-1} . The bands observed with visible excitation do not appear, although a weak shoulder appears in the vicinity of 1600 cm^{-1} .

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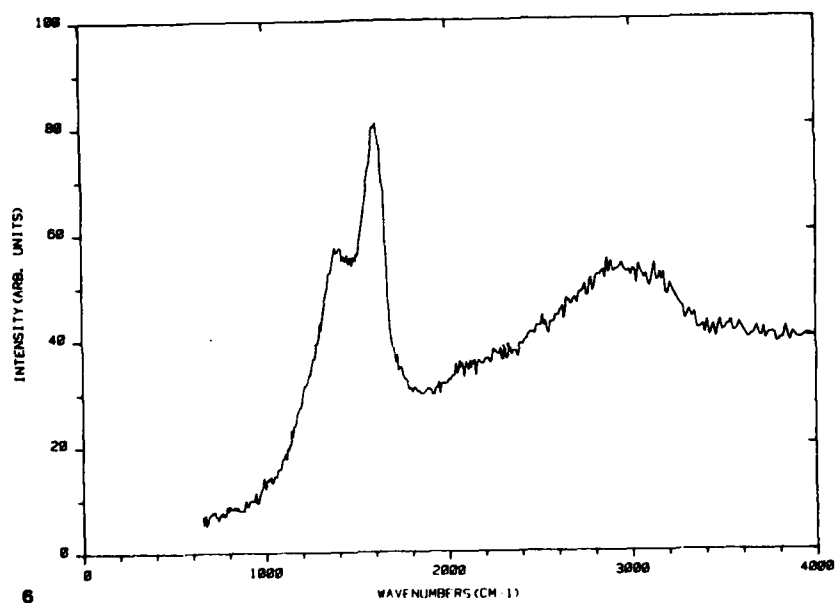


Figure 6. Raman spectrum of melanin suspended in a KBr pellet. The sample was excited at 488.0 nm, 100 mW laser power on the sample. The spectrum is an average of 30 sweeps from 600 cm^{-1} at 10 cm^{-1} per second. The instrument time constant was 0.1 sec. The spectral band pass was 5 cm^{-1} .

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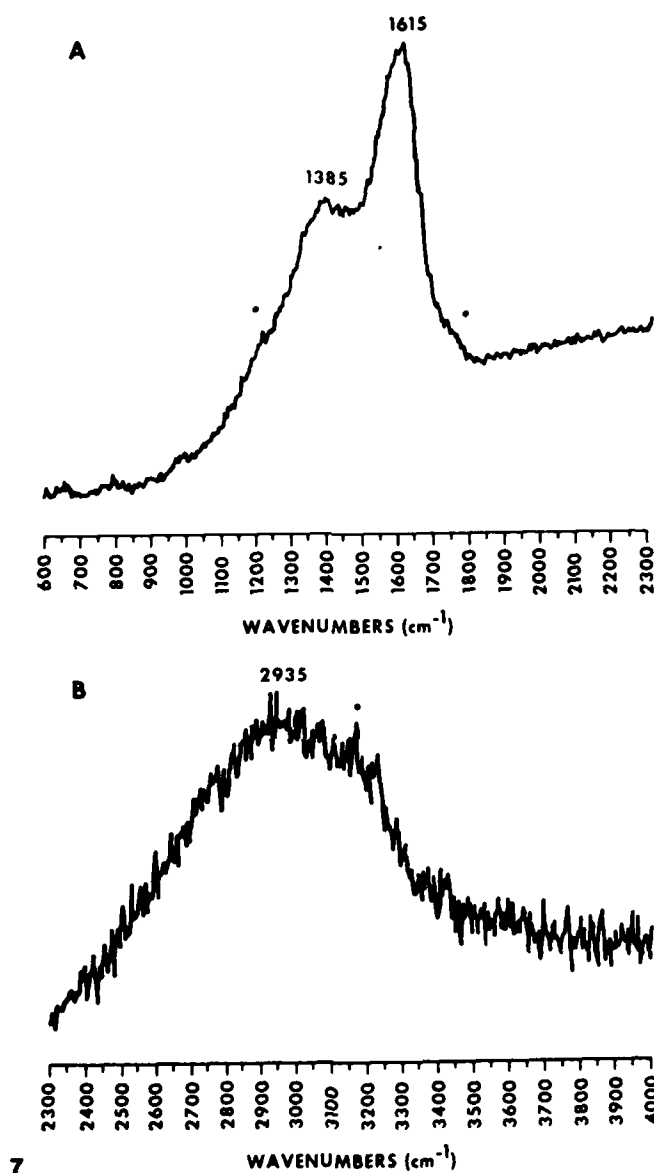
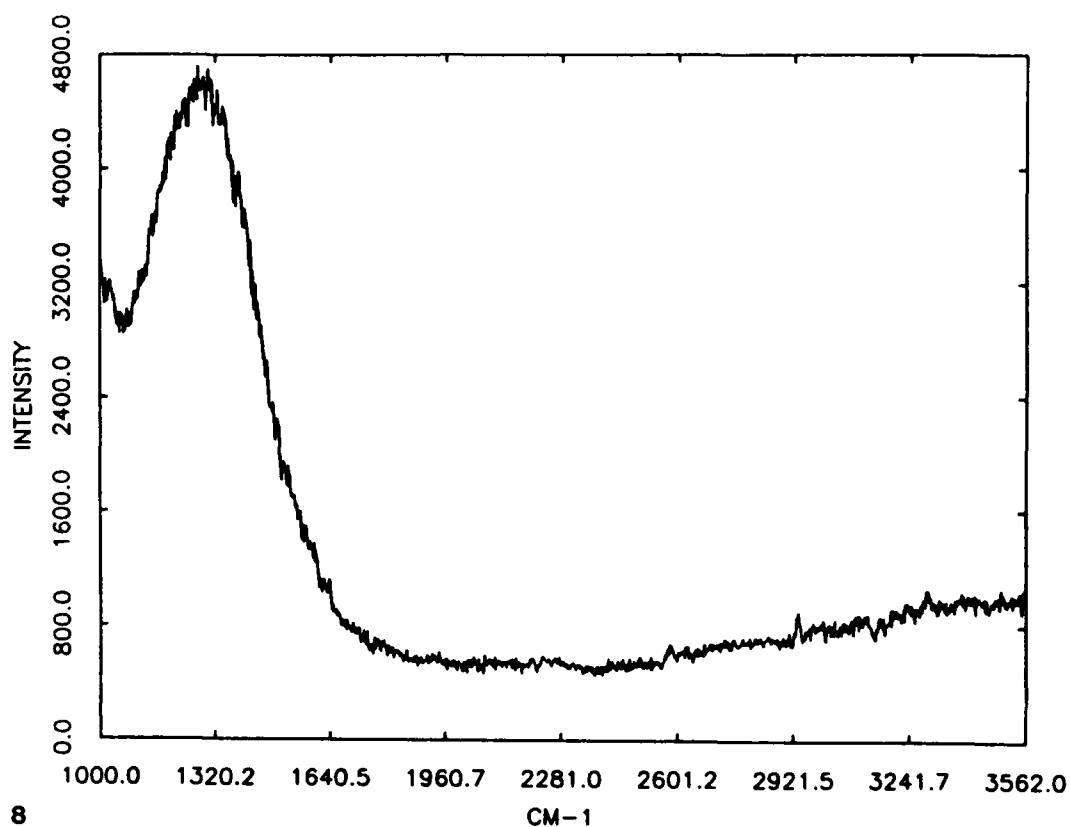


Figure 7. Raman spectra of melanin in specific regions. The melanin samples were suspended in a KBr pellet (0.6% (w/w)). The pellet placed on a rotating sample platform was excited at 488.0 nm, 100 mW laser power on the sample. The spectral band pass was 5 cm^{-1} and the instrument time constant was 0.1 sec. Spectrum A is Raman data obtained from melanin from $600\text{--}2300 \text{ cm}^{-1}$. The spectrum is an average of 70 sweeps. Bands labeled with an asterisk (1250 cm^{-1} , 1750 cm^{-1}) are weak shoulders that did not resolve to distinct peaks. Spectrum B is the Raman data obtained from 2300 to 4000 cm^{-1} . The spectrum is an average of 70 sweeps. The shoulder marked with an asterisk (3170 cm^{-1}) did not resolve to a distinct peak.

Spectroscopic Studies of Melanin

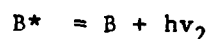
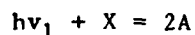


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Figure 8. Ultraviolet Raman spectrum of melanin suspended in a KBr pellet. The sample was excited at 363.8 nm with a power of 5mW on the sample. The spectrum was collected in a step mode, 2 cm^{-1} steps, 50 sec integration time per step. The spectral band pass was 5 cm^{-1} .

DISCUSSION

The original goal of this study was the observation of Raman scattering from melanin. In order to learn the conditions whereby the fluorescent background was small enough to make the measurement of Raman scattering possible, we studied in detail the luminescence of melanin obtained from argon ion laser excitation. The results presented in Figure 2 indicate that the chemiluminescence observed in melanin is connected with a second order process. The simple experiment performed indicates that upon illumination an intermediate is rapidly produced which then undergoes a dimerization reaction according to the mechanism



The species X undergoes a first order photodissociation process to two A species. These recombine in a second order process to form an excited species B* which then fluoresces to ground state B. The direct proportionality of the intensity of the phototransient as a function of input laser power indicates that the production of A is directly proportional to the laser power, as would be expected from the first step of the reaction above. Steps two and three of the reaction scheme follow the work of Slawinski et al. (15), (16), who also observed a second order dimerization reaction in the measurement of the chemiluminescence in the photo-oxidation of humic acids.

The scheme presented above, the complex photokinetics are further complicated by slow photodegradation reactions occurring on a longer timescale. For this reason, the flow system was employed so that a steady-state signal could be obtained which would not change during the time course of collection of data.

The steady state chemiluminescence spectra obtained showed features similar to those results obtained by Lakowicz and Keating-Nakamoto (18), who observed red-edge excitation of fluorescence in proteins and membranes. They discussed a two-state model of fluorescent chromophores where one population of chromophores resides in a hydrophobic, solvent-inaccessible region of the polymer, the other accessible to the solvent. The chromophore is first promoted to an initially excited state. Polar solvent effects tend to red-shift the fluorescence. If the rate of solvent relaxation is substantially less than the rate of fluorescence, then red-shifted fluorescence will only be observed when the solvent substantially interacts with the chromophore. If the rate of solvent relaxation is faster than the rate of fluorescence, then the emission spectrum will

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be solvent-shifted at any excitation wavelength. According to Figure 4, the wavenumber of maximum emission is independent of the laser excitation line in the vicinity of 21839 cm^{-1} . As the excitation line approaches 19435 cm^{-1} , the emission maximum red-shifts. This trend continues to excitation at 632.8 nm (15803 cm^{-1}). It appears that the rate of fluorescence greatly exceeds the rate of solvent relaxation. At lower energy excitation, solvation significantly affects the emission spectrum. The shape of the emission spectrum remains the same, with the full width at half maximum never changing. The intensity of the emission drops dramatically as the excitation energy lowers. This decrease results from a decrease in the population of chromophores capable of attaining an excited state. Excitation of the red edge of the absorption spectrum selects that population of chromophores existing in a polar environment where the energy of the excited state is lowered by solvent interactions.

The only samples from which Raman spectra could be obtained were melanin samples embedded in KBr pellets. The presence of KBr, and perhaps the absence of water, minimized the fluorescence background to the point where Raman scattering could be observed. The intensity of fluorescence in KBr pellets varied from sample to sample. Melanin from a freshly opened bottle had little fluorescent background when illuminated in a KBr pellet. Melanin from older samples tended to have a higher fluorescent background. Most likely this effect results from air oxidation and the binding of water to melanin. Indeed, supporting this observation, Kozikowski et al. (14) observed that melanin oxidized by hydrogen peroxide has a higher fluorescence than unoxidized melanin.

The major features observed from the Raman spectrum of melanin are peaks at 1385 cm^{-1} , $1590\text{--}1615\text{ cm}^{-1}$ and 2930 cm^{-1} , shoulders at 1250 , 1750 , and 3170 cm^{-1} upon argon ion laser excitation, and an intense band at 1300 cm^{-1} upon UV excitation. It is instructive to compare these results with infrared absorption data obtained from melanin in KBr pellets (8). In the carbonyl stretch region there is a band at 1615 cm^{-1} (carboxylate anion antisymmetric stretch), 1715 cm^{-1} protonated carboxylate carbonyl stretch and a broadened absorption from $1000\text{--}1500\text{ cm}^{-1}$, peaking at 1350 cm^{-1} , resulting from carboxylate ion symmetric stretch. The positions of the Raman bands are similar, indicating that the carboxylate anion contributes to the Raman signal. It is possible that some of the 1590 cm^{-1} to 1615 cm^{-1} band results from aromatic ring stretch modes (19). The Raman band at 1385 cm^{-1} is a combination of carboxylate anion symmetric stretch and ring stretch modes. The shoulder at 1250 cm^{-1} is an aromatic C-C stretch mode and the shoulder at 1750 cm^{-1} is a carbonyl stretch mode. The band at 2935 cm^{-1} is a C-H stretch mode and the band at 3170 cm^{-1} is an OH stretch mode.

Upon UV laser excitation, the Raman spectrum of melanin changes

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drastically, with one major band observed at 1301 cm^{-1} . The changes in the observed Raman spectrum from excitation at 488.0 nm to 363.8 nm indicate that resonance enhancement occurs in this system. The excitation spectrum of melanin fluorescence shows a maximum at 390 nm (D.L. Bolton, unpublished results). It appears that UV laser excitation selectively enhances vibration modes associated with an electronic transition, most likely an aromatic π - π^* transition. A peak at 1300 cm^{-1} could be due to ring vibrations of the fluorescing chromophore in melanin. It is also possible that a phototransient may be observed by this technique. Rigorous assignment of the melanin Raman bands requires systematic variation of experimental conditions and observation of spectroscopic effects. An enhancement profile of the Raman spectra of melanin is presently being measured in this laboratory.

The true nature of the excited state of melanin is not understood. A survey of the literature indicates that no unifying model exists for the behavior of melanin. For example, the absorption spectrum of melanin fits to an amorphous semiconductor model (8), yet since it is found in biological systems, the methods previously used to study melanin have involved biochemistry and biology and to a lesser degree optical spectroscopy and semiconductor physics.

The present study has focused on the chemiluminescence and Raman scattering from tyrosine melanin. Much of the spectral behavior involves fluorescence and resonance enhanced Raman scattering from an aromatic species present in the polymer. Because of the amorphous semiconductor properties of melanin, it is not known how much the observed spectral behavior involves the conduction band. Is the fluorescent species a localized state, or is its electron cloud delocalized through extensive conjugation with the rest of the polymer, as would occur in a valence to conduction band transition?

The data presented in this paper give an indication of the complexity of melanin and the requirement for a broad, multidisciplinary approach to their properties. We have observed chemiluminescence from melanin involving second order kinetics. The behavior of the spectra as the excitation wavelength is varied is indicative of species strongly interacting with the solvent, whereby the energy of a polar excited state is lowered by solvent interactions. Luminescence was observed at all exciting wavelengths.

The presence of melanin in biological pigments and its role in photoprotection in the eyes and skin make study of its excited state properties necessary for knowledge of its biological function. Changes in its excited state properties may have some role in Parkinsonism or melanomas. The shape of the excitation spectrum presented in Figure 5 is remarkably similar to the blue light sensitivity curve for photochemical (20) injury to the eye presented by Marshall (21). These results imply that melanin may be a component

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of the photochemical eye damage mechanisms involving blue and UV light. In contrast, the curve presented in Figure 5 is steeper than the photochemical action spectrum measured for oxygen consumption by melanin (12), (22).

The Raman data demonstrate the existence of carboxylate anions in melanin. Such negatively charged species will bind cations and positively charged species like paraquat (23). The change in the appearance of the Raman spectrum upon UV excitation implies that a UV absorbing species produces resonance enhanced Raman scattering upon UV excitation. Observation of Raman scattering from melanin in solution may be possible through the use of coherent antistokes Raman scattering. It is possible that this species is implicated in the blue light damage mechanism and has clinical relevance in Parkinsonism and melanomas.

CONCLUSIONS AND RECOMMENDATIONS

Chemiluminescence kinetics obtained from synthetic tyrosine melanin were of a second order decay process. The full width at half maximum of the steady state emission spectrum was independent of the excitation line. The position of the emission maximum was independent of excitation wavelength at short wavelength excitation (near 457 nm), but the emission maximum varied at longer wavelength excitation (greater than 514.5 nm). As the excitation wavelength was varied from 465.8 nm to 514.5 nm, emission intensity dropped by a factor of 6. The Raman spectra revealed broad bands at 1385, 1590-1615, and 2930 cm^{-1} . However, after ultraviolet laser radiation (363.8 nm) a single broad band appeared at 1300 cm^{-1} . The UV effect implies a resonance enhancement from an UV absorbing chromophore. In addition, our results stress the complexity of the excited state species of melanin and therefore the potential complexity of biologic function. Knowledge gained about the excited states of melanin may provide a better understanding of the importance that melanin plays in human and animal tissues. Since the absorption spectrum of melanin closely fits an amorphous semiconductor model, it is recommended that additional studies be performed utilizing the principles and methods of amorphous semiconductor physics as well as exploration of its properties at high pressure.

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